An-Najah Univ. J. Res., Vol. 15, 2001

Effect of Indomethacin Suppository on Serum Glucose, Some Lipids, Non-Protein Nitrogen Constituents and Rectal Mucosa of Rabbit

تأثير لبوس الاندوميثاسين على سكر الجلوكوز وبعض اللبيدات والمكونات النيتروجينية غير البروتينية في السيرم وعلى الغشاء المخاطي لمستقيم الأرنب .

Ismail I. S. Abdel Aziz

Department of Biology, Faculty of Science, Islamic University, Gaza, Palestine.

Received: (18/4/1999), Accepted: (5/11/2001)

Abstract

The present study was conducted to investigate the side effects of the therapeutic doses of anti-inflammatory drug indomethacin suppository for 20 days on serum non-protein nitrogen constituents e.g., urea, uric acid, creatinine, glucose, cholesterol and triglycerides in addition to the structure of the rectal mucosa of rabbit. Twenty-four male rabbits weighing (1800-2000 gm) were used in this study. They were divided into four equal groups. The first group served as control. In the second and the third groups of rabbits, indomethacin suppository is given to each rabbit in a dosage levels including 100 mg of indomethacin per day for 10 and 20 days respectively, while the fourth group of animals received the same previous dose of indomethacin for 20 days and then stopped for 2 weeks to study the possibility of recovery.

The daily rectal administration of indomethacin suppository for 20 days increased significantly the concentration of glucose, urea, uric acid, creatinine and triglycerides, in rabbit's serum. However, cholesterol contents did not show any significant changes. The histological results of treated rabbits had produced noticeable pathogenic consequences in the rectal mucosal cells. The severity of such pathological lesions is obviously time dependent. There is a severe rectal mucosal damage characterized by generalized severe villous atrophy, focal desquamation of the rectal epithelium and the goblet cells, were markedly swollen and the lamina propria showed degeneration with hemorrhagic foci as well as noticeable inflammatory cellular infiltration. The nuclei of the epithelial cells showed obvious nuclear damage with distinct signs of pyknosis. Lymphocytic infiltration was also detected within the mucosa. However, condition after this drug was stopped these rectal mucosal cells showed some signs of regeneration.

ملخص

يهدف هذا البحث إلى دراسة التأثيرات الجانبية التي تحدثها الجرعات العلاجية لعقار الأندوميثاسين - كلبوس شرجي - المضاد للالتهابات لمدة عشرين يوماً على سكر الجلوكوز وبعض اللبيدات والمكونات النتروجينية غير

البروتينية مثل اليوريا وحمض البوليك والكرياتينين والكولسترول والجلسريدات الثلاثية وكذلك على الغشاء المخاطي لمستقيم الأرنب.

وقد استخدم في هذا البحث أربع وعشرون من ذكور الأرانب التي يتراوح وزن كل منها ١٨٠٠-٢٠٠٠ جرام، حيث قسمت الحيوانات إلى أربع مجموعات متساوية العدد واعتبرت المجموعة الأولى للمقارنة، أما المجموعة الثانية والثالثة فقد تم إدخال لبوس شرجي يحتوي على ١٠٠ مليجرام من الأندوميثاسين لمدة عشرة وعشرون يوماً على التوالي، أما المجموعة الرابعة فقد أعطيت نفس الجرعة السابقة ولمدة عشرين يوماً ثم أوقف إعطاء العقار وتركت لمدة أسبوعين لدراسة إمكانية الاستشفاء من الآثار الجانبية لهذا العقار.

وقد أوضحت الدراسة أن هناك زيادة ملموسة في تركيز الكرياتينين واليوريا وحمض البوليك والجلوكوز بالإضافة إلى الجلسريدات الثلاثية، وقد كان لعقار الأندوميثاسين تأثير قليل على مستوى الكولسترول في سيرم الأرانب. أما في مجموعة الاستشفاء فقد تلاشت بعض التغيرات في القياسات السابقة نتيجة توقف إعطاء العقار.

أما من الناحية النسيجية فقد أظهرت الفحوص تكسير واضمحلال الخلايا المبطنة لجدار الطبقة المخاطية وكذلك الخلايا المخاطية الكأسية. كما حدث تهدم للنسيج الضام المكون للطبقة التحت مخاطية مع وجود نزف دموي متدرج وتجمع للخلايا الالتهابية في هذه الطبقة وبزيادة زمن التجربة لوحظ حدوث تتكرز لهذه الخلايا وتحلل كروماتيني لأنويتها وتفكك وتحلل شديدين في النسيج الضام الرابط لها. وفي مجموعة الاستشفاء من هذا العقار لوحظ تلاشي بعض هذه التغيرات سابقة الذكر، مما يدل على استعادة جدار المستقيم المخاطي لحالته ولكن بصورة غير كاملة.

Introduction

The nonsteroidial ant-inflammatory agents (NSAIA) are chemically heterogeneous compounds that have therapeutic and toxic effects in common (Higgs et al., 1984).

NSAIA inhibit the enzyme cyclooxygenase, which catalyzes the conversion of arachodonic acid to prostaglandins and thromboxane (Seibert et al., 1994). Cyclooxygenase is directly inhibited by nonsteriodal anti – inflammatory drugs including salicylates, indomethacin, and ibuprogen. Drugs under development inhibit more distal steps in the cycloxygenase and lipooxygenase pathways. However, the fact that a drug inhibits the synthesis of a certain eicosanoid does not necessarily mean that a given effect of the drug is the direct result of a

deficiency of that eicosanoid. Most currently available drugs inhibit early reactions in the synthetic pathways and therefore block the formation of more than one product. Indomethacin not only inhibits formation of cyclic endoperoxides by cyclooxygenase but also disrupts calcium flux across membranes, inhibits cyclic adenosine monophosphate (cyclic AMP) – dependent protein kinase and plosphodiesterase, and inhibits one of the enzymes responsible for degradation of PGE2 (Roberston 1981).

Some studies have shown that aspirin and salicylates readily produce acute tubular necrosis and renal papillary necrosis in animals (Aranoled et al., 1973, Axelsen, 1976; and Prescott, 1982). Faragalla et al., (1993) found that paracetamol and /or velosef induced many degenerative changes and diminution of the glycogen contents in the liver cells of the albino rats.

As regards the possible mode of action of these drugs, Nichander et al., (1979), reported that they inhibit lysosomal membrane labilization. Furthermore, NSAIA influence mucopolysaccharides biosynthesis in normal and inflamed tissue (Shen and Winter, 1977).

Abnormal liver function tests after usage of nonsteroidal anti-inflammatory agents has been detected by some investigators (Paulus et al., 1987) and Magdalou et al., (1990). Only few cases of acute renal failure and nephrotic syndrome in patients taking priprofen were reported by Domenget et al., (1984).

Creatinine is known to be formed in the body exclusively from creatine via creatine phosphate. Increases of creatinine are the graver prognostic significance’s than those other nitrogenous substances and their estimation has sometimes been used for diagnosis of renal disease. These substances together with urea, form the major part of the non-protein nitrogen of the blood which is sometimes determined instead of urea (Varley, 1976).

Nevertheless, some investigators as a consequence of using several anti-inflammatory drugs have reported certain side effects. The commonest unwanted effects include intestinal ulceration, erosion, exfoliation,

perforation and bleeding, together with secondary anemia resulting from blood loss in such cases (Robert, 1981 and Whittle and Vane, 1983).

The present work was planned to demonstrate the side effects of one dose of the anti-inflammatory drug "Indomethacin" for different periods on some biochemical parameters of blood and the structure of the rectal mucosa of rabbit. The possibility of recovery and reattaining of the normal condition after drug withdrawal was also investigated.

Materials and Methods

Twenty-four of male rabbits weighing about 1800-2000 gm were used. They were fed on a commercial balanced diet prepared especially for rabbits (Anber). The diet and tap water were offered ad libitum all over the experimental period.

Rabbits were divided into four sub groups, (each containing 6 rabbits) as follows:

Sub group 1: kept as a control.

Sub group II: One suppository 100 mg of indocid was inserted daily into the rectum for 10 days.

Sub group III: Each rabbit was daily rectally administered with the same dose of drug for 20 days.

Sub group IV: Each rabbit was daily rectally administered with the same dose of the drug for 20 days and killed after two weeks following the last dose to test for any reversible changes.

The anus of rabbits was closed immediately after insertion, with a thick plaster for one hour to prevent any leakage.

At the end of the experiment, blood samples from experimental and control animals were collected directly from jugular vein as recommended by Shakoori et al. (1992). Clear serum samples were separated by centrifugation at 3000 r.p.m. for 20 min. and then collected and stored in a deep freeze at (-20^{o} C) for different biochemical analysis.

However, determination of enzymes was carried out on fresh serum samples.

Serum samples were analyzed for glucose, triglycerides and total cholesterol by the methods described by Trinder (1969), Fossati and Prencipe (1982) and Allain (1974), respectively.

Non-protein nitrogen constituents were determined by the methods of Mackay and Mackay (1927) for urea, Fossati et al., (1980) for uric acid and Bartels and Bohmer (1972) for creatinine.

Specimens of the rectum were taken and processed for light microscopic examination, 7u thick paraffin sections were stained with haematoxylin and eosin (Drury and Wallington1980).

Using SPSS performed the statistical analysis for T-test.

Results and Discussion

Results in table (1), represent the effect of daily rectal administration of indomethacin suppository for 20 days on the non-protein nitrogen constituents of rabbits serum i.e. urea, uric acid and creatinine.

Urea concentration in rabbit's serum treated with indomethacin suppository for 10 days and 20 days increased significantly by 19.2% and 22.3% while in recovering rabbit it was 19.7% in comparison with the control level (Table1). Urea is the principal end product of protein catabolism. Enhanced protein catabolism and accelerated amino acid deamination for gluconeogensis is probably an acceptable postulate to interpret the elevated levels of urea. The presence of some toxic compounds might increase blood urea and decrease plasma protein (Varely, 1976). In addition, the elevation of blood urea is a good indicator for kidney diseases, and may indicate renal damage.

In general, uric acid content in rabbit's blood serum increased in response to the rectal administration of indomethacin suppository for 20 days (Table 1). Uric acid concentration in the serum of treated rabbit's in the tenth and twentieth days of treatment were 1.5 and 1.6 times, respectively, greater than the control level. While in recovering rabbit it

was 1.4 times, greater than control level. Uric acid is the end product of the catabolism of tissue nucleic acids i.e., Purine and Pyrimidine bases metabolism (Wolf et. al., 1972). In the present study, the serum uric acid levels exhibited significant increment in the treated rabbits for 10 days and 20 days. This may be due to degradation of purines and pyrimidines or to an increase of uric acid level by either over production or inability of excretion (Wolf et. al., 1972).

Table 1: Effect of Indomethacin Suppository on some of the Chemical Constituents of Rabbit's Serum.

Parameters	Control n=6	Experimental groups		
		10 days n=6	20 days n=6	Recovery n =6
Urea (mg/d1)	30.83±1.89	38.17±1.14*	39.67±0.88*	38.33±0.49*
Uric acid (mg/d1)	0.75±0.11	1.10±0.11*	1.20±0.08*	1.07±0.08*
Creatinine (mg/d1)	0.90±0.07	1.26±0.06*	1.44±0.09*	1.13±0.06
Glucose (mg/d1)	124.17±4.78	127.0± 4.79	138.50±4.46*	121.67±4.36
Cholesterol (mg/d1)	57.50±3.84	54.50± 4.11	50.50±4.39	52.83±3.93
Triglycerides (mg/dl)	133.33±5.02	149.17±5.09*	158.33±5.44*	150.0±3. 42*

All values expressed as mean ± SE

* Significant differences at $P < 0.05$

Creatinine concentration in rabbit's serum treated with indomethacin suppository for 10 days and 20 days increased significantly by 28.6% and 37.5% while in recovering rabbit it was 20.4% in comparison with the control level. Creatinine is the least variable nitrogenous constituent of the blood, it is more readily excreted by the kidneys than urea and uric acid. Serum creatinine concentration is only elevated when kidney function is seriously impaired. About 50% of kidney function must be

lost before a rise in the serum concentration of creatinine can be detected (Kaptan and Szasbo, 1983).

Rectal administration of indomethacin for 10 and 20 days increased serum glucose contents of the treated rabbits by 2.3 % and 10. 3 %, while in recovering rabbit it was 2.1 % compared to the control level (Table 1). Indomethacin may directly or indirectly play a specific role in pancreatic secretions, gluconeogensis process, glycogen metabolism or glucose oxidation. It means that there was a disturbance in carbohydrate metabolism of rabbits by the daily rectal administration of indomethacin.

Cholesterol concentration in blood serum of treated rabbit's in the tenth and twentieth days of treatment were 1.06 and 1.14 times respectively, lower than the control level (Table 1). While in recovering rabbit it was 1.09 times lower than the control level.

The statistical analysis of the data table (1), showed that serum triglycerides content of the rabbits treated with indomethacin at the different levels, increased significantly at the tenth and twentieth days post treatment. Triglycerides content in the serum of the rabbit's treated with indomethacin suppository for 10 days and 20 days increased significantly by 10.6% and 15.8% while in recovering rabbit it was 11.1% in comparison with the control level. The changes observed in serum triglycerides in response to the treatment by indomethacin suppository, take place in the liver due to imbalance between the normal rates of lipid synthesis, utilization and secretion (Glaser and Mager, 1972) .The increment in serum triglycerides content agreed with that reported by Mohammed and Steitia.,(1995) who found an increase in the number of lipid droplets in mice liver treated with diclofenac sodium.

The rectal mucosa of control rabbits was thrown into folds. The surface epithelium was formed of columnar cells, which have basal oval nuclei and pale acidophilic cytoplasm. Among these columnar cells, few goblet cells were observed. The lamina propria exhibited loose texture with few connective tissue fibers and cells. The rectal glands were of simple tubular type. They were lined with columnar as well as numerous goblet cells (Fig. 1).



Figure 1: Rectal mucosa of control rabbit. showing normal folding, low columnar epithelium. The lamina propria (L.P.) consists of loose connective tissue and contained scattered rectal glands, (G).
(H&E x125).

Treatment of rabbit with daily 100 mg of indomethacin for ten days produced certain pathological consequences in the rectal mucosal cells of such treated animals. The surface mucous cells have designated noticeable damage and erosion. There was some swelling of both absorptive and goblet cells. The lamina propria has also undergone a certain degree of damage and injury expressed mainly by an apparent increase of lymphocytic cells as seen in figure (2).

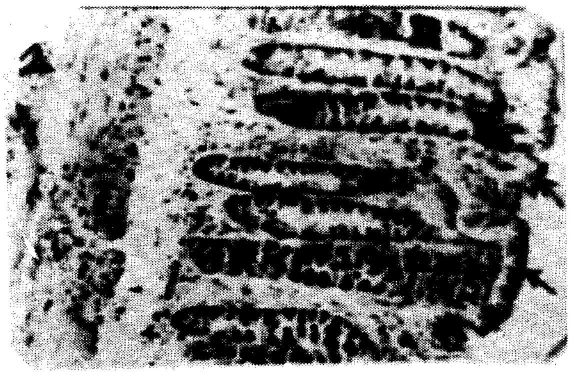


Figure 2: Rectal mucosa of treated rabbit after application of indomethacin suppository for 10 days showing obvious degeneration of the lining epithelial cells.
(H&E x125).

Treatment of rabbit with daily 100 mg of indomethacin for 20 days produced more remarkable injury of the rectal tissues as illustrated in figure (3). This figure reveals that the mucosal lining epithelium was obviously detached from the underlying basement membranes. The mucosal columnar cells have designated obvious vacuolated cytoplasm and distinct pyknotic nuclei. The lamina propria showed cellular infiltration mainly of plasma cells and eosinophils especially around blood vessels. The glands were enlarged and distended with secretion and their lining cells were swollen (Fig. 3).



Figure 3: Rectal mucosa of treated rabbit after application of indomethacin suppository for 20 days showing severe rectal damage, the goblet cells were markedly swollen, the lamina propria degenerated and the nuclei of the epithelial cells showed nuclear damage. Lymphocytic infiltration was also detected within mucosal area.

(H&E x125).

The mucosal lesions are in conformity with those obtained by Shea et al., (1990); Hawkey et al., (1991) and Nakmura et al., (1992) using several non-steroidal anti-inflammatory drugs.

An explanation of the marked deleterious consequences on the rectal mucosal cells following treatment with indomethacin drug is believed to

be due to the direct effect of the drug, which inhibits the synthesis, and production of the mucous protective barrier. Bhattacharge et al., (1991) have illustrated that the gastric mucosal erosion and ulceration which accompanied the use of several non-steroidal anti-inflammatory drugs appeared to be a consequence of attenuated gastric mucin activity and reduction in the gastric mucoprotective barrier.

The marked necrotic lesions displayed in the rectal mucosal cells and the lamina propria are significantly related to the damage of both superficial and deeper microvessels. Those include rupture of the capillary walls, necrosis of endothelial cells, damage to endothelial cells organelles, deposition of fibrin and adherence of platelets to damaged endothelium as illustrated by Tarnawski et al., (1990) in case of rats treated with aspirin.

Nevertheless, Mescheter et al., (1990) showed that phenylbutazone caused duodenal mucosal changes in the horses in the form of sloughing of the surface epithelium with sub-surface capillaries and microthrombosis with large numbers of neutrophils together with abundant fibrin and cellular debris at the erosion sites.

In the present study the degeneration of lamina propria was also clearly illustrated in a more or less similar manner to what had previously been described by Bugat et al., (1976) in the gastrointestinal mucosal cells of rats intoxicated with lysin-o-acetyl salicylate and aspirin, respectively.

This degenerative feature could also be attributed to the damage of blood supply as well as impairment of tissue respiration. From previously introduced data it is obvious that the administration of indomethacin for long periods induces deleterious side effects on the histological structure of the rectal mucosa.

Animals treated with 100 mg of indomethacin for 20 days and then left untreated for two weeks showed that the surface epithelial cells were partially regenerated after two week of drug withdrawal, there was some focal areas which still exhibited obvious symptoms of damage erosion (Fig. 4). The lamina propria have manifested obvious regression of their

injurious alterations with lesser vacuolation and reduced lymphocytic infiltration.

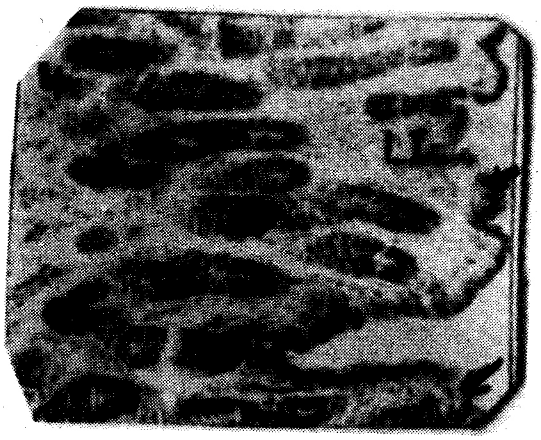


Figure 4: Rectal mucosa of treated rabbit after the withdrawal of indomethacin suppository, showing some improvement and repair of the villar epithelium. The component tissues of the lamina propria (L.P.) manifested obvious lesser vaculation with reduced lymphocytic infiltration. (IC).

(H&E x125).

Two weeks after stopping the drug, the possibility of recovery of the rectal mucosa is noticed. Similar recovery was reported by Bravo et al., (1992) in the gastric mucosa of rats after exposure of different damaging agents including aspirin and ethanol. In this regard, Levi et al., (1992) illustrated that there was a regenerative response in patients with duodenal ulcers occurring in the absence of NSAIAS therapy.

The present results revealed that the incorporation of indomethacin suppository merely lead to some capillary dilation and perivascular cell infiltration. Rectal glands were also dilated and showed swollen mucous cells. The capillary dilation is a sign of active absorption of the drug, whereas the cellular infiltration may point to a reactional process due the presence of the absorbed drug in the intestinal tissue (Nouth et al., 1988).

References

1] Allain, C.C.; Poon L. S.; Chan C.S.G.; Richmond, W. and Fu, P.C. (1974): Enzymatic determination of total serum cholesterol. Clin. Chem. 20 (4): 470-75.

2] Arnold, I.; Collins, C. and Starmer, G.A. (1973): The short-term effects of acetylsalicylic acid in the rat kidney. Further observations. Nephron. 10: 355.

3] Axelsen, R.A. (1976): Analgesic-induced renal papillary necrosis in the gunn rat. The comparative nephrotoxicity of asprin and phenacelin. J. Pathol. 120:145.

4] Bartels, H. and Bohmer, M. (1972): Clin. Chem. Acta. 37:193.

5] Bhattacharya, S.K.; Goel, P.K; Bhattocharya, S.K. and Tandon, R. (1990): Potentiation of gastric toxicity of ibuprofen by paracetamol in the rat. J. Pharmacol. 43 (7): 520-521.

6] Bravo, M.L.; Escolar, G.; Navarro, C.; Fontarnau, R. and Bulbena, Q. (1992): Morphological study of gastric lesions developing in the rat under several damaging conditions: Modifications induced by pre treatment with Zinc acexamate. Scanning Microsc., 6 (3): 855-862 .

7] Bugat, A.M.; Thompson, R.; Aures, D. and Grossman, I.M. (1976): Gastric mucosal lesions produced by intravenous infusion of aspirin in cats. Gastroenterology, 71, (6): 764-759.

8] Domenyget, J.F.; Laseur, M.; Blanc, P. and Foliani, J. (1984): Acute renal failure and nephrotic syndrome related to pirporfen. Press Med., 13 (5): 283-284.

9] Durury,R.A.B. and Wallington,E.A.(1980):Carletons histological technique,5th ed., Oxford Univ.Press.

10] Farag–Alla, A.M and El-Negmy, F.A. (1993): Effect of Paracetamole and/or Velosef treatments on the glycogen content of albino rat liver. Egypt. J. Histol. 16 (2): 407-415.

11] Fossati, P. Prencipe, L., and Berti G. (1980) Clin. Chem. 26/2, 227.

12] Fossati, P.; Prencipe, L. (1982): Serum triglcerides determined colorimetrically with an enzyme that produces hydrogen peroxide, Clin. Chem.; 28 (10): 2077-08.

13] Glaser, G. and Mager, J. (1972): Biochemical studies on the; mechanism of liver poisons. II. Induction of fatty liver. Biochem. Biophys. Acta 261:500.

14] Hawkey, C.J.; Hawthorne, A.B.; Hudson, N.; Cole, A.T. Mohida, Y.R. and Daneshmend, T.K. (1991): Separation of the impairment of haemostasis by aspirin from mucosal injury in the human stomach. Clin. Soci. 81 (4): 565-573.

15] Higgs,G. (1984): Non. Steriodal anti-inflammatory drugs. In textbook of immunopharmacology; edited by Dole, M.M. and Foreman, J.C.; P. 305-314. Blackwell Scientific Publication, Oxfor, London, Edinburgh.

16] Kaptan, A. and Szabo, L.L. (1983): Clinical Chemistry: Interpretation and techniques. Second edition.

17] Levi, S.; Goolldlad, R.A.; Lee, C.Y.; Stamp, G.; Walport, M.J.; Wright, N.A. and Hodgson, H.J. (1992): Non-steroidal anti-inflammatory drugs inhibit the processes of mucosal cell proliferation associated with duodenal ulcer healing. Digestion, 53 (3-4): 129-133 .

18] Mackay, E.M. and Mackay. L.L. (1927): The concentration of urea in the blood of normal individuals. Clin. Invest. 4:295.

19] Magdalou, J.; Chjes, V.; Lafaurie, C and Siest, G. (1990): Glucoronidation of Z-arylpropionic acids: pirprofen, flurbiprofen and ibuprofen by liver microsomes. Drug metab. Dispos. Biol. Fate. Chem., 18 (5): 692-697.

20] Mohamed A. Sana, Steitia M. Ferial (1995): Effect of diclofinac sodium on the structure of the mice liver; light and electron microscopical study. Egypt, J.Histol. 19(1): 249-262.

21] Nakamura, J.; Katayama, M.; Ishida, K. and Sasaki, H. (1992): An assessment of indomethacin-induced mucosal damage in vivo by measuring the metabolism of salicylamide in rabbit intestine. Chem. Pharm. Bull. Tokyo, 40 (5): 1261-1265.

22] Nouth, A.T.; Ramadan, E.M. and Abdel-Gawad, A.H. (1988): Formulation of azapropazone suppositories and examination of the effect on the GIT of rabbits. Pharm. Ind. 50: 750.

23] Paulus, H.E.; Furst, Dromgoole, S.H. (1987): Drugs for rheumatic disease. Churchill Livingstone; New York, Edinburgh, London, Melbourne, 421-422.

24] Prescott, L.F. (1982): Analgesic nephropathy: A reassessment of the role of phenacetin and other analgesics. Drugs: 23:75.

25] Robert, A. (1981): A current history of cytoprotetion. International work-shop on protective actions of prostaglandins on the gastrointestinal mucosa. Prostaglandins, 21. (Suppl). 89-96.

26] Seibert-K; Zhang – Y; Leahy – K; Hauser-S; Masferrer- J; Perkins – W; Lee- L; Isakson-P (1994): Pharmacological and biochemical demonstration of the role of cyclooxygenase 2 in inflammation and pain- Proc-Natl-Acad – Sci – U. S. A. 91 (25): 1203: 7.

27] Shea, D.T., Steel, L.; Montcalm, M.E. and Dubois, A. (1990): Aspirin-induced changes in gastric function role of endogenous prostaglandins and mucosal damage. Gastroenterology. 98 (2): 248-292.

28] Shen, T. and Winter, C.A. (1977): Chemical biological studies on Indomethacin, silindac and their analogues. In: Advances in drug research; edited by Haprer, N.J. and Simmonds. A.B.; Vol. 12, P. 28-245; Academic Press. New York.

29] Tarnawski, A.; Stachura, J.; Gergely, H. and Holadner, D. (1990): Gastric microvascular endothelium: A major target for aspirin-induced injury and arachidonic acid protection. An ultrastructual analysis in the rat. Eur. J. Clin. Invest., 20 (4): 432-440.

30] Trindeer, P. (1969): Determination of glucose in blood glucose oxidase with an alternative oxygen acceptor. Ann. Clin. Biochem 6: 24-27.

31] Varley, H. (1976): Practical Clinical Chemistry (4th Edition) Published by Galab Kazinrani for Arnold-Heineman, Naraina Phase 1, New Delhi, 28. Biochemistry. (Non Protein Nitrogen): 190.

32] Whittle, B.J.R. and Vane, J.R. (1983): Prostacyclin, thromboxanes and prostaglandins- action and roles in the gastrointestinal tract. In progress in Gastroenterollogy, Grune and Stratton. Inc. New York, PP3-30.

33] Wolf, P.L.; Williams, D.; Tsudaka, T. and Acosta, L. (1972): Methods and Techniques in Clinical Chemistry. Wiley-Interscience adivison of John Wiley and Sons, Idnc., New York, London, Sydney, Toronto .